

iGEM 2016: Pittsburgh
Week 2 Lab Notebook

Tuesday, May 31

Chemical Hygiene training

[Transformation](#) to [test competency](#) (50, 20, 10, 5 chloramphenicol plates)

Wednesday, June 1

Competent Cell Test Kit

Cells grew on all plates-- CM problem?

Pour LB and CM [plates](#) (polymerized well)

Thursday, June 2

[Cell free extract reaction](#)

T7-GFP (28 ng/uL)

uL	25	20	15	10	5
Solution A (40%)	10	8	6	4	2
Solution B (30%)	7.5	6	4.5	3	1.5
RNase (.5%)	1.25	1	.75	.5	.25
Nuclease free H2O	--	--	--	--	--
DNA (250 ng)	8.93	7.14	5.36	3.57	1.8
	27.68	22.14	16.61	11.07	5.55

25 uL rxn vol	250 ng	200 ng	150 ng	100 ng	50 ng	Control
DNA	8.93	77.14	5.36	3.57	1.79	See June 3
H2O	--	--	.89	2.68	4.46	
Solution A	10	10	10	10	10	
Solution B	7.5	7.5	7.5	7.5	7.5	
RNase	1.25	1.25	1.25	1.25	1.25	
rfu*	209.38		361.96		298.45	

Only ran 250, 150, and 50 ng reactions due to limited DNA

*Wavelength (485/585 nm, excitation/emission) --not correct peak

Competent Cell Test Kit

Re-plate Competent Cell Test Kit cells on LB and CM plates
New transformations: 50 and 10
4 plates: 6/2 50 (20 uL), 6/2 10 (20 uL), 5/31 5 & 50 (10 & 10 uL), 5/31 20 & 10 (10 & 10 uL)

OUTREACH

Tissue engineering camp (Praneeth)

Pitt Science Outreach -- scirocks@pitt.edu, Geard Fossett

Carnegie Science Center

Online form

BrownJ@CarnegieScienceCenter.org -- Jason Brown, Director of Science and Education)

OrtenzoL@CarnegieScienceCenter.org -- Linda Ortenzo, Director of STEM Programs

Related Programs Needing Volunteers

The Citizen Science Lab -- AndreSamuel@urbaninnovation21.org ("Teach")

The Ellis School -- Brooke McLane-Higginson, mclane-higginsb@theellisschool.org

Urban Impact -- amanda.wagner@uifpgh.org, Camp Manager

Friday, June 3

Cell free extract reaction: control

25 uL rxn volume	0 ng
Solution A	10 uL
Solution B	7.5
RNase	1.25
H2O	6.25
DNA	0
rfu	1.35

Too much RNase in all reactions -- should be .125 uL

Cell competency test kit (grow longer)

5/31 5: 1 colony

6/2 50: 1

6/2 10: 4

Efficiency = colonies/1 uL * pg/uL * 20 uL/251 uL * $1 \cdot 10^{-6}$ ug/pg = $3.5 \cdot 10^{-5}$ cfu/ug

OUTREACH

PSOC -- Barbara Paul -- community events and YMCA's